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DEVELOPMENT OF AN EMERGENCY COMPATIBILITY (CROSSMATCH) TEST

ANNUAL REPORT

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April, 1978

Supported by

**U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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SUMMARY

When red cells are suspended in saline of lower ionic strength (LISS) than usual (e.g., 0.03 M instead of 0.17 M) the rate of antibody association is enhanced. Studies were performed to evaluate the use of LISS for compatibility tests. We evaluated: optimal ionic strength; optimal serum/red cell ratio and incubation time; sensitivity compared with methods used nationally at present; and non-specific reactions. 0.03 M NaCl + 0.3 M glycine was established as the optimal LISS. Slightly enhanced reactivity was obtained if equal volume of serum and 1 volume of RBC. After only 5 minutes incubation in LISS 67% of antiglobulin reactive weak antibodies were detected, compared with only 33-36% in saline or albumin.

When 30 weak red cell alloantibodies were tested, 90% were detected after only 10 minutes incubation in LISS compared with 80% after 30, and only 70% after 15 minutes in saline or albumin (examples of Rh, Duffy, and Kidd antibodies were not detected). The three antibodies missed by LISS were very weak examples of anti-Le^a which were retested x2 later and found to be weakly reactive in LISS. When 100 antibodies of varying characteristics were tested, agglutinating antibodies were generally found to not be enhanced by LISS but all of the antiglobulin reactive antibodies reacted equally or better with LISS suspended cells. Non-specific reactions have not been a problem to date. LISS appears to have significant advantages over current compatibility test procedures.

BACKGROUND

It has been common practice for many years to carry out compatibility testing on red cells suspended in normal (0.9%) saline (NaCl). The most commonly used procedures involve incubating donor red cells and recipient serum at room temperature and 37C. Bovine albumin is often added and after the tubes are inspected for agglutination the red cells are washed and tested with antiglobulin serum (indirect antiglobulin test). Incubation times are not standard, but usually times such as those recommended by the AABB Technical Methods and Procedures Manual (1) are used, i.e., 15-30 minutes at room temperature followed by 15-30 minutes at 37C. It was reported that sensitization of cells in bovine albumin medium, prior to the addition of the antiglobulin reagent, enhanced the strength of the reaction above that of the traditional method using saline (2). From this study it was concluded that a 15 minute sensitization period of albumin-suspended cells, with sera containing antibodies, is equal to a longer time of incubation in either medium when followed by the antiglobulin procedure.

In 1964, Hughes-Jones et al (3) and Elliot et al (4) showed that if the ionic strength of the red cell suspending medium was lowered the antiglobulin reaction was considerably enhanced. The speed of reaction can be increased 1000-fold by a reduction in the salt concentration from 0.17 M to 0.03 M (5). Although these reports suggested from the detection of most blood group antibodies was enhanced, and indeed, suggested that sensitivity equaled that of using enzyme-treated red cells, the principles have not been generally utilized in manual testing although they have been employed in automated antibody testing (6).

However, one study (7) has been reported using a low ionic strength solution as a red cell diluent as a routine method in a large blood bank, and the results appear very promising. Following institution of the low ionic strength method, the total number of antibodies detected increased, and the reactions were more clear-cut and easier to interpret. An incubation time of only 5 minutes was employed. More than 100,000 units of blood crossmatched using this method have been transfused without any transfusion reactions due to unidentified blood group antibodies. Unfortunately, parallel studies utilizing low ionic strength solution and saline were not reported, the only controls consisting of a comparison with results of the previous year.

Two further studies have been published, utilizing a low ionic strength medium for antibody detection (8,9). Both of these studies suggested that low ionic strength solutions were advantageous in antibody detection.

The main advantages of LISS seem to be: 1) A generally agreed upon shortening of the incubation time and 2) There are indications

in the literature that some antibodies may be detected by LISS that are missed in the regular saline system. Some of these antibodies belong to the group generally considered as being clinically important (e.g., Rh and Kidd) but others are considered clinically insignificant (e.g., Bg).

The disadvantages of LISS may be 1) Non-specificity under certain conditions (e.g., ionic strength of solution too low causing non-specific aggregation of non-sensitized red cells; non-specific uptake of complement under certain conditions (10); non-specificity associated with certain anti-human sera. 2) Enhancement of clinically insignificant antibodies (e.g., cold auto-antibodies (I), Bg, etc). 3) Increase in thermal range of some cold antibodies (e.g., some anti-M that react only at room temperature with saline suspended cells will react at 37C with LISS suspended cells. 4) One report shows decreased sensitivity in the detection of Lewis antibodies. 5) Instability of LISS (without preservatives).

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READING (GRADING) OF AGGLUTINATION RESULTS FOR TOTAL STUDY

All tests were inspected macroscopically and all negative reactions were checked microscopically. The following gradings were used.

- 4 \equiv ++++ No agglutinated cells - background clear*
- 3 \equiv +++ Several large agglutinates - few free cells - background clear*
- 2 \equiv ++ Moderate size agglutinates - more free cells - background slightly cloudy*
- 1 \equiv + Numerous small agglutinates - many free cells - background cloudy*
- *Visible Macroscopically
- $\frac{1}{2}$ \equiv \pm Scattered agglutinates in a sea of unagglutinated cells**
- 0 No agglutination.

**Usually visible macroscopically

If () is used it indicates a slightly weaker reaction - e.g., (1) is weaker than 1.

$\frac{1}{2}$ = intermediate reaction - e.g., $2\frac{1}{2}$ is stronger than 2 but weaker than 3.

I. DETERMINATION OF OPTIMUM IONIC STRENGTH FOR RED CELL DILUENT

- a) Low ionic strength solutions (LISS) were prepared using 0.17 M NaCl diluted in varying amounts of 0.3 M solution sodium glycinate. Osmolality of all solutions was measured and recorded.
- b) A pilot study was performed on 20 normal sera containing no antibodies by saline, albumin, enzyme or antiglobulin techniques, and 20 weak antibodies (e.g., ABO, Rh, K, Fy^a, Jk^a) using red cells suspended in NaCl solutions with molarities ranging from 0.01 - 0.17.

An ionic strength that gave no false-positive reactions with the normal sera and gave optimal reactions with the antibodies was selected for the rest of the study.

PREPARATION OF LOW IONIC STRENGTH SOLUTIONS (LISS)

	pH	<u>Sodium Chloride Molarity</u>						
		0.17	0.10	0.07	0.04	0.03	0.02	0.01
Saline (0.17 M)	6.3	980	590	410	236	180	118	59 ml
PO ₄ Buffer (0.15 M)	6.7	20	20	20	20	20	20	20 ml
Sodium Glycinate (0.3 M)	6.7	-	390	570	744	800	862	921 ml
Osmolality		316	308	304	302	300	298	295 ml
pH		6.4	6.6	6.7	6.8	6.8	6.8	6.9

RESULTS

Normal Serum X Day Area Panel #022577

a) Agglutination at 37°C (30 minutes incubation)

SALT SOLUTIONS

	0.01	0.02	0.03	0.04	0.07	0.10	0.17
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0

b) Indirect Antiglobulin Test at 37°C (30 minutes incubation)

	<u>SALT SOLUTIONS</u>						
	0.01	0.02	0.03	0.04	0.07	0.10	0.17
1	$\underline{+}^m$	0^s	0	0^s	0^s	0	0
2	$(\underline{+})^m$	0^s	0^s	0^s	0^s	0	0
3	(1)	$\underline{+}^m$	0	0	0	0	0
4	1	$\underline{+}^m$	$\underline{+}^m$	0^s	0^s	0^s	0^s
5	$\underline{+}^m$	0^s	0	0^s	0	0	0
6	$\underline{+}^m$	$(\underline{+})^m$	0	0	0	0^s	0
7	(1) ^m	$(\underline{+})^m$	0	0^s	0^s	0	0
8	$\underline{+}^m$	0^s	0^s	0	0	0^s	0
9	0^s	0^s	0	0	0	0	0
10	0^s	0^s	0	0	0	0	0
11	$(\underline{+})^m$	$(\underline{+})^m$	0	0	0	0	0
12	0^s	0^s	0	0	0	0	0
13	$(\underline{+})^m$	0^s	0	0	0	0	0
14	$(\underline{+})^m$	$(\underline{+})^m$	0	0	0	0	0
15	$(\underline{+})^m$	$(\underline{+})^m$	0^s	0^s	0	0	0
16	0^s	0^s	0	0^s	0	0	0
17	$(\underline{+})^m$	$(\underline{+})^m$	0	0	0	0	0
18	0^s	$(\underline{+})^m$	0	0	0	0	0
19	0^s	0^s	0^s	0	0^s	0	0
20	0^s	0	0	0	0	0	0

m = microscopically positive

s = "sticky", i.e., a few cells sticking together,
no definite agglutination

II. Serum Containing Alloantibodies X Bay Area Panel #022577

a) Agglutination at 20°C (30 minutes incubation)

	<u>SALT SOLUTIONS</u>						
	0.01	0.02	0.03	0.04	0.07	0.10	0.17
1. Anti-A	4	4	4	4	4	4	4
2. Anti-B	4	4	4	4	4	4	4
3. Anti-H	1	1	1	(1)	(1)	(1)	(1)
4. Anti-N	(1)	(1)	(1)	$\frac{1}{2}$	0	0	0
5. Anti-Le ^a	(1)	(1)	(1)	(1)	(1)	(1)	(1)
6. Anti-P ₁	3	3	3	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$

b) Agglutination at 37°C (30 minutes incubation)

	<u>SALT SOLUTIONS</u>						
	0.01	0.02	0.03	0.04	0.07	0.10	0.17
7. Anti-K	0	0	0	0	0	0	0
8. Anti-K	0	0	0	0	0	0	0
9. Anti-Jk ^a	0	0	0	0	0	0	0
10. Anti-Jk ^a	0	0	0	0	0	0	0
11. Anti-Fy ^a	0	0	0	0	0	0	0
12. Anti-Fy ^a	0	0	0	0	0	0	0
13. Anti-Fy ^a	0	0	0	0	0	0	0
14. Anti-Fy ^a	0	0	0	0	0	0	0
15. Anti-D	0	0	0	0	0	0	0
16. Anti-C	0	0	0	0	0	0	0
17. Anti-E	0	0	0	0	0	0	0
18. Anti-c	0	0	0	0	0	0	0
19. Anti-e	0	0	0	0	0	0	0
20. Anti-Le ^a	0	0	0	0	0	0	0

II. Serum Containing Alloantibodies X Bay Area Panel #022577

c) Indirect Antiglobulin Test at 37°C (30 minutes incubation)

	<u>SALT SOLUTIONS</u>						
	0.01	0.02	0.03	0.04	0.07	0.10	0.17
7. Anti-K	1½	1½	2	2	2	2	1½
8. Anti-K	2½	2½	2½	2½	2½	2	2½
9. Anti-Jk ^a	1½	1½	1½	1½	1	1	(1)
10. Anti-Jk ^a	1	1	1	1	1	1	1
11. Anti-Fy ^a	1	1	1	1	1	1	1
12. Anti-Fy ^a	1	1	1	1	1	1	1
13. Anti-Fy ^a	(1)	(1)	(1)	(1)	(1)	(1)	(1)
14. Anti-Fy ^a	1½	1½	1½	1	1	1	1
15. Anti-D	2	2	2	2	2	2	1½
16. Anti-C	(1)	(1)	(1)	(1)	(1)	(1)	(1)
17. Anti-E	1½	1½	2	1	1	1	1
18. Anti-c	3	3	3	3	3	3	3
19. Anti-e	1½	1½	1½	1	1	(1)	(1)
20. Anti-Le ^a	(1)	(1)	(1)	(1)	(1)	(1)	(1)

Summary of Results of Part I

Salt solutions of different ionic strengths were prepared by diluting buffered saline with sodium glycinate solutions to produce solutions with sodium chloride molarities varying from 0.01 to 0.17.

20 normal sera were then tested for their ability to agglutinate normal red cells after incubation at 37C in each of these solutions. Normal sera did not cause direct agglutination of normal red cells in any of the solutions tested.

The 20 normal sera were then tested against normal red cells by the indirect antiglobulin test after incubation at 37C. Microscopically positive results were obtained with salt solutions of sodium chloride molarity of 0.01 and 0.02. Although there were occasional "sticky" reactions using solutions of molarity of 0.03 and higher there was only one microscopically positive reaction at molarity of 0.03.

Next, 20 sera containing antibodies were tested. Antibodies tested included Anti-A, -B, -H, -N, -Le^b, -P₁, -K, -Jk^a, -Fy^a, -D, -C, -E, -e, -c and -Le^a. The results of direct agglutination at 37C were negative throughout. Results of direct agglutination at 20C and of the indirect antiglobulin test at 37C revealed slight but definite augmentation of the reactions particularly in solutions of sodium chloride molarity of 0.03. The 0.03 M solution was selected for further studies.

2. DETERMINATION OF OPTIMAL INCUBATION PERIOD

30 weak antibodies (antibodies were selected that gave 2+, or less, reactions by conventional techniques using saline suspended cells) were tested against red cells suspended in saline (0.17 M), 30% bovine albumin or low ionic strength solution (LISS) by indirect antiglobulin test.

A recently published study (Moore, H.C. and Mollison, P.L., Transfusion 16:291, 1976) has shown that is important to use equal volumes of LISS suspended red cells and serum rather than the conventional 2 volumes of serum to 1 volume of 5% red cells, (to avoid affecting the ionic strength of the LISS). Therefore, the following techniques were used in the comparative study.

Indirect Antiglobulin Test (IAT)

Saline (Sal): 2 volumes of serum were added to a 10x75mm glass tube and 1 volume of 5% red cells suspended in 0.17 M saline were added. Tests were either washed x4 in 0.17 M saline immediately or incubated at 37C for 5 minutes, 10 minutes, 15 minutes, 30 minutes or 60 minutes before washing.

Albumin (Alb): Same as saline test but 2 volumes of 30% bovine albumin were added to the serum before red cells were added.

Low Ionic Strength Solution (LISS): 2 volumes of serum were added to a 10x75 mm glass tube and 2 volumes of 2% red cells were added. Tests were either washed x4 in 0.17 M immediately, or incubated at 37C for 5 minutes, 10 minutes, 15 minutes, 30 minutes, or 60 minutes before washing.

Six sera were tested by direct agglutination, using the same red cell diluents, volumes, and incubation periods as above. The tests were centrifuged immediately, or following incubation, and read for agglutination.

Notes: All red cells used were washed x3 in normal saline before being resuspended in 0.17 M saline or LISS. Some of the weak antibodies were obtained by diluting antibodies in inert normal serum.

As an extra volume of red cells were used in the LISS method a weaker cell suspension (2%) was used in order to keep the final antigen antibody approximately equal in all three methods.

DIRECT AGGLUTINATION: INCUBATION TIME
(MINS) AT 24°C

	0	5	10	15	30	60
<u>Anti-A</u>						
1. Sal	4	4	4	4	4	4
Alb	4	4	4	4	4	4
LISS	4	4*	4*	4*	4*	3½*
<u>Anti-B</u>						
1. Sal	4	4	4	4	4	4
Alb	4	4	4	4	4	4
LISS	4	4*	4*	3½*	4*	3½*
<u>Anti-M</u>						
1. Sal	0	0	0	0	0	0
Alb	0	0	0	(1)	(1)	(1)
LISS	0	1	2	2	2	2
<u>Anti-N</u>						
1. Sal	(1)	1½	2	2½	3	3
Alb	0	(1)	(1)	2	2	2
LISS	(1)	1½	2	2½	3	3
<u>Anti-P₁</u>						
1. Sal	(1)	2	3	3	3	3
Alb	0	1½	2	2	2	2
LISS	(1)	2	3	3	3	3
<u>Anti-Le^b</u>						
1. Sal	0	1	1½	1½	2	2
Alb	0	1	1	1	1½	1½
LISS	0	1	1½	1½	2	2

* Lysed (2+)

INDIRECT ANTIGLOBULIN TEST
INCUBATION TIMES (MINS)

	0	5	10	15	30	60
<u>Anti-K</u>						
1. Sal	1	1	1½	1½	1½	1½
Alb	1	1	1½	1½	1½	1½
LISS	1	1½	1½	1½	1½	1½
2. Sal	(1)	1	1½	1½	2	2
Alb	(1)	1½	1½	2	2	2
LISS	(1)	1½	1½	2	2	2
3. Sal	½	1	1½	1½	2	2
Alb	½	1	1½	1½	2	2
LISS	½	1½	1½	1½	2	2
4. Sal	0	(1)	1	1	1	1
Alb	0	(1)	1	1	1	1
LISS	0	(1)	1	1	1½	1½
5. Sal	½	1	1	1	2	2
Alb	(1)	1	1	1	2	2
LISS	(1)	1	1	1	2	2
6. Sal	0	(1)	(1)	(1)	1	1
Alb	0	(1)	(1)	(1)	1	1
LISS	0	(1)	(1)	(1)	1	1
<u>Anti-k</u>						
1. Sal	0	0	0	(1)	(1)	(1)
Alb	0	0	½	(1)	(1)	(1)
LISS	0	(1)	(1)	1	1	1
<u>Anti-Jk^a</u>						
1. Sal	0	0	0	(1)	(1)	(1)
Alb	0	0	0	(1)	(1)	(1)
LISS	0	0	(1)	(1)	1	1
2. Sal	0	0	0	0	0	1
Alb	0	0	0	0	(1)	1
LISS	0	0	1	1	1½	1½
3. Sal	0	0	0	(1)	(1)	(1)
Alb	0	0	(1)	(1)	(1)	(1)
LISS	0	½	(1)	1	1	1
4. Sal	0	0	(1)	1	1	1
Alb	0	0	(1)	1	1	1
LISS	0	(1)	1	1½	1½	1½

INDIRECT ANTIGLOBULIN TEST
INCUBATION TIMES (MINS)

	0	5	10	15	30	60
<u>Anti-Jk^a</u>						
5. Sal	0	1	2	2½	2½	2½
Alb	0	1	2	2½	2½	2½
LISS	0	2½	2½	2½	2½	2½
<u>Anti-Fy^a</u>						
1. Sal	0	0	0	½	½	½
Alb	0	0	0	½	(1)	(1)
LISS	0	(1)	(1)	1	1	1
2. Sal	0	0	0	0	0	½
Alb	0	0	0	0	0	½
LISS	0	0	(1)	(1)	(1)	(1)
3. Sal	0	0	0	(1)	(1)	(1)
Alb	0	0	(1)	(1)	(1)	(1)
LISS	0	(1)	1	1	1	1
4. Sal	0	0	0	½	(1)	(1)
Alb	0	0	0	½	(1)	(1)
LISS	0	(1)	1½	1½	1½	1½
5. Sal	0	0	0	(1)	(1)	1
Alb	0	½	(1)	(1)	(1)	1
LISS	0	1	1	1	1	1
6. Sal	0	½	(1)	1	1	1½
Alb	0	½	(1)	1	1½	1½
LISS	0	1	1	1	1½	1½
<u>Anti-Fy^b</u>						
1. Sal	0	0	0	0	0	(1)
Alb	0	0	0	0	0	(1)
LISS	0	1	1	1	1	1
<u>Anti-Rh</u>						
<u>Anti-D</u>						
1. Sal	0	0	(1)	1	1½	1½
Alb	0	0	(1)	1	1½	2½
LISS	0	0	2	2	2	2½

**INDIRECT ANTIGLOBULIN TEST
INCUBATION TIMES (MINS)**

	0	5	10	15	30	60
<u>Anti-Rh</u>						
<u>Anti-D</u>						
2. Sal	0	0	0	0	0	0
Alb	0	0	0	$\frac{1}{2}$	(1)	(1)
LISS	0	0	(1)	1	1	1
3. Sal	0	0	0	0	0	1
Alb	0	0	0	0	0	1
LISS	0	(1)	$1\frac{1}{2}$	$1\frac{1}{2}$	$1\frac{1}{2}$	$1\frac{1}{2}$
<u>Anti-C</u>						
1. Sal	0	0	0	0	0	0
Alb	0	0	0	0	0	$\frac{1}{2}$
LISS	0	0	(1)	(1)	(1)	(1)
<u>Anti-E</u>						
1. Sal	0	0	0	0	0	0
Alb	0	0	0	0	(1)	(1)
LISS	0	0	1	1	1	1
<u>Anti-c</u>						
1. Sal	0	(1)	1	1	2	2
Alb	0	(1)	$1\frac{1}{2}$	$1\frac{1}{2}$	2	2
LISS	0	1	2	2	2	2
<u>Anti-e</u>						
1. Sal	0	0	0	0	$\frac{1}{2}$	(1)
Alb	0	0	0	$\frac{1}{2}$	(1)	1
LISS	0	(1)	(1)	(1)	(1)	1
<u>Anti-Le^{a*}</u>						
1. Sal	0	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$
Alb	0	0	0	0	0	0
LISS	0	0	0	0	0	0
2. Sal	0	0	0	(1)	(1)	(1)
Alb	0	0	0	0	0	0
LISS	0	0	0	0	0	0

*An equal volume of fresh Le(a-b) serum was added to all sera as a source of fresh complement.

**INDIRECT ANTIGLOBULIN TEST
INCUBATION TIMES (MINS)**

	0	5	10	15	30	60
Anti-Le^{a*}						
3. Sal	0	(1)	1	1	1	1½
Alb	0	(1)	1	1	1	1½
LISS	0	(1)	1	1	1	1
4. Sal	0	0	0	½	(1)	(1)
Alb	0	0	0	0	½	½
LISS	0	0	0	0	0	0

SUMMARY OF INDIRECT ANTIGLOBULIN TESTS

1. Number (Percentage) of Antibodies Detected By IAT Following Each Incubation Period in Various Diluents

	<u>Red Cell Diluent</u>		
<u>Incubation Period</u>	<u>Saline</u>	<u>Albumin</u>	<u>LISS</u>
No incubation	2 (7%)	2 (7%)	2 (7%)
5 minutes	10 (33%)	11 (36%)	20 (67%)
10 minutes	12 (40%)	16 (53%)	27 (90%)
15 minutes	20 (67%)	21 (70%)	27 (90%)
30 minutes	24 (80%)	24 (80%)	27 (90%)
60 minutes	27 (90%)	28 (93%)	27 (90%)

2. Antibodies Not Detected By Each Method At Various Incubation Periods

<u>Incubation Period</u>	ANTI-										
	K(6)	k(1)	Jk ^a (5)	Fy ^a (6)	Fy ^b (1)	D(3)	C(1)	E(1)	c(1)	e(1)	Le ^a (4)
0 Mins											
Sal	2	1	5	6	1	3	1	1	1	1	4
Alb	2	1	5	6	1	3	1	1	1	1	4
LISS	2	1	5	6	1	3	1	1	1	1	4
5 Mins											
Sal	0	1	4	5	1	3	1	1	1	1	3
Alb	0	1	4	4	1	3	1	1	1	1	3
LISS	0	0	2	1	0	2	1	1	0	0	3
10 Mins											
Sal	0	0	3	5	1	2	1	1	0	1	3
Alb	0	0	2	3	1	2	1	1	0	1	3
LISS	0	0	0	0	0	0	0	0	0	0	3
15 Mins											
Sal	0	0	1	1	1	2	1	1	0	1	2
Alb	0	0	1	1	1	1	1	1	0	0	3
LISS	0	0	0	0	0	0	0	0	0	0	3
30 Mins											
Sal	0	0	1	1	1	2	1	1	0	0	0
Alb	0	0	0	1	1	1	1	0	0	0	2
LISS	0	0	0	0	0	0	0	0	0	0	3
60 Mins											
Sal	0	0	0	0	0	1	1	1	0	0	0
Alb	0	0	0	0	0	0	0	0	0	0	2
LISS	0	0	0	0	0	0	0	0	0	0	3

SUMMARY OF RESULTS OF PART 2

The use of weak antibodies for the tests demonstrated significant increases in sensitivity when LISS was used in contrast to normal saline or albumin. After only 5 minutes incubation at 37C, 67% of the weak antibodies were detected using LISS suspended red cells, whereas only 33-36% were detected using saline or albumin. At 10 minutes, 90% of the antibodies were detected in LISS compared to 40-53% in saline or albumin. The number of antibodies detected by LISS did not increase, even after 1 hour incubation; three examples of weak anti-Le^a not being detectable at any incubation period. After 60 minutes incubation, two of the anti-Le^a were not detected using albumin either, but were detected using saline suspended cells. Three examples of weak Rh antibodies, anti-C, anti-E and anti-c, were not detected by saline suspended red cells even after one hour of incubation. These same Rh antibodies were detected after 10 minutes using LISS and using albumin suspended cells one was detected at 10 minutes, two at 30 minutes and all three at 1 hour.

It is interesting to note that after 15 minutes incubation in albumin, which is one of the most commonly used procedures for compatibility testing in the United States, only 70% of the very weak antibodies were detected; 1 anti-Jk^a, 1 anti-Fy^a, 1 anti-Fy^b, 3 anti-Rh and 3 anti-Le^a were not detected. In contrast, at 10 or 15 minutes only the 3 very weak anti-Le^a were missed by the LISS technique. These three anti-Le^a were tested x2 on different days following the initial testing and were then found to react very weakly with LISS suspended cells. If these results are substituted then the LISS techniques detected 100% the antibodies at 10 minutes.

3. False-Positive Reactions Using LISS

- a) Sera from 100 normal blood donors and 100 unselected hospital patients antibodies by conventional screening methods were retested in parallel with LISS. All tests were incubated at room temperature and then 37C, read for agglutination and sensitization detected by the anti-globulin test. All negative reactions were checked microscopically.
- b) Complement components and indeed gamma globulin are known to be bound to red cells by non-immune mechanisms under certain low ionic strength conditions. Therefore, 20 of the sera from a) were retested using monospecific antiglobulin sera at the antiglobulin phase (e.g., anti-IgG, -IgM, -IgA, -C3, -C4). As there is considerable variation in the amount of anti-C-3 and -C4 present in commercial broad-spectrum reagents and the amount of anti-C3 may be increased in future products, the monospecific anti-C-3 and -C4 used will be at a concentration greater than that present in any commercial reagent at present.

False-Positive Reactions using LISS

(a) 100 Normal donors

		RT		37C		IAT			RT		37C		IAT		
		SAL	ALB	SAL	ALB	SAL	ALB		LISS	LISS	LISS	LISS	LISS	LISS	
1		0	0	0	0	0	0		0	0	0				
2		0	0	0	0	0	0		0	0	0				
3		0	0	0	0	0	0		0	0	0				
4		0	0	0	0	0	0		0	0	0				
5		0	0	0	0	0	0		0	0	0				
6	EZZE	0	0	0	0	0	0		0	0	$\frac{1}{2}$				
7		0	0	0	0	0	0		0	0	0				
8		0	0	0	0	0	0		0	0	0				
9		0	0	0	0	0	0		0	0	0				
10		0	0	0	0	0	0		0	0	0				
11		0	0	0	0	0	0		0	0	0				
12		0	0	0	0	0	0		0	0	0				
13		0	0	0	0	0	0		0	0	0				
14		0	0	0	0	0	0		0	0	0				
15		0	0	0	0	0	0		0	0	0				
16		0	0	0	0	0	0		0	0	0				
17		0	0	0	0	0	0		0	0	0				
18		0	0	0	0	0	0		0	0	0				
19		0	0	0	0	0	0		0	0	0				
20		0	0	0	0	0	0		0	0	0				
21		0	0	0	0	0	0		0	0	0				
22		0	0	0	0	0	0		0	0	0				
23		0	0	0	0	0	0		0	0	0				
24		0	0	0	0	0	0		0	0	0				
25		0	0	0	0	0	0		0	0	0 ^s				
Cells: group O segments, washed 3x in Saline, 1x in LISS															
Coombs' serum, Pfizer 73051															

False-Positive Reactions using LISS

(a) 100 Normal donors

		RT		37C		IAT		RT		37C		IAT	
		SAL	ALB	SAL	ALB	SAL	ALB			LISS	LISS	LISS	
26		0	0	0	0	0	0		0	0	0		
27		0	0	0	0	0	0		0	0	0		
28		0	0	0	0	0	0		0	0	0		
29		0	0	0	0	0	0		0	0	0		
30		0	0	0	0	0	0		0	0	0 ^s		
31		0	0	0	0	0	0		0	0	0		
32		0	0	0	0	0	0		0	0	0		
33		0	0	0	0	0	0		0	0	0		
34		0	0	0	0	0	0		0	0	0		
35		0	0	0	0	0	0		0	0	0		
36		0	0	0	0	0	0		0	0	0		
37	DUNC	0	0	0	0	0	0		0	0	(1)		
38		0	0	0	0	0	0		0	0	0		
39		0	0	0	0	0	0		0	0	0		
40		0	0	0	0	0	0		0	0	0		
41		0	0	0	0	0	0		0	0	0		
42		0	0	0	0	0	0		0	0	0		
43		0	0	0	0	0	0		0	0	0		
44		0	0	0	0	0	0		0	0	0		
45		0	0	0	0	0	0		0	0	0		
46		0	0	0	0	0	0		0	0	0		
47		0	0	0	0	0	0		0	0	0		
48		0	0	0	0	0	0		0	0	0		
49		0	0	0	0	0	0		0	0	0		
50		0	0	0	0	0	0		0	0	0		
Cells: group O segments, washed 3x in Saline, 1x in LISS													
Antiglobulin serum: Pfizer 73051													

False-Positive Reactions using LISS

(a) 100 Normal donors

		RT		37C		IAT			RT		37C		IAT		
		SAL	ALB	SAL	ALB	SAL	ALB		LISS	LISS	LISS	LISS	LISS	LISS	
51		0	0	0	0	0	0		0	0	0	0	0	0	
52		0	0	0	0	0	0		0	0	0	0	0	0	
53		0	0	0	0	0	0		0	0	0	0	0	0	
54		0	0	0	0	0	0		0	0	0	0	0	0	
55		0	0	0	0	0	0		0	0	0	0	0	0	
56		0	0	0	0	0	0		0	0	0	0	0	0	
57		0	0	0	0	0	0		0	0	0	0	0	0	
58		0	0	0	0	0	0		0	0	0	0	0	0	
59		0	0	0	0	0	0		0	0	0	0	0	0	
60		0	0	0	0	0	0		0	0	0	0	0	0	
61		0	0	0	0	0	0		0	0	0	0	0	0	
62		0	0	0	0	0	0		0	0	0	0	0	0	
63		0	0	0	0	0	0		0	0	0	0	0	0	
64		0	0	0	0	0	0		0	0	0	0	0	0	
65		0	0	0	0	0	0		0	0	0	0	0	0	
66		0	0	0	0	0	0		0	0	0	0	0	0	
67		0	0	0	0	0	0		0	0	0	0	0	0	
68		0	0	0	0	0	0		0	0	0	0	0	0	
69		0	0	0	0	0	0		0	0	0	0	0	0	
70		0	0	0	0	0	0		0	0	0	0	0	0	
71		0	0	0	0	0	0		0	0	0	0	0	0	
72		0	0	0	0	0	0		0	0	0	0	0	0	
73		0	0	0	0	0	0		0	0	0	0	0	0	
74		0	0	0	0	0	0		0	0	0	0	0	0	
75		0	0	0	0	0	0		0	0	0	0	0	0	
Cells: group O segments, washed 3x in Saline, 1x in LISS															
Antiglobulin Serum: pfizer 73051															

False-Positive Reactions using LISS

(a) 100 Normal donors

		RT		37C		IAT			RT		37C		IAT		
		SAL	ALB	SAL	ALB	SAL	ALB		LISS	LISS	LISS	LISS	LISS	LISS	
76		0	0	0	0	0	0		0	0	0				
77	WALK	0	0	0	0	0	0		0	0	1 1/2				
78		0	0	0	0	0	0		0	0	0				
79		0	0	0	0	0	0		0	0	0				
80		0	0	0	0	0	0		0	0	0				
81		0	0	0	0	0	0		0	0	0				
82		0	0	0	0	0	0		0	0	0				
83		0	0	0	0	0	0		0	0	0				
84		0	0	0	0	0	0		0	0	0 ⁵				
85		0	0	0	0	0	0		0	0	0				
86		0	0	0	0	0	0		0	0	0				
87		0	0	0	0	0	0		0	0	0				
88		0	0	0	0	0	0		0	0	0				
89		0	0	0	0	0	0		0	0	0				
90		0	0	0	0	0	0		0	0	0				
91		0	0	0	0	0	0		0	0	0				
92	MILL	0	0	0	0	0	0		0	0	(1)				
93		0	0	0	0	0	0		0	0	0				
94		0	0	0	0	0	0		0	0	0				
95		0	0	0	0	0	0		0	0	0				
96		0	0	0	0	0	0		0	0	0				
97		0	0	0	0	0	0		0	0	0				
98		0	0	0	0	0	0		0	0	0				
100		0	0	0	0	0	0		0	0	0				
		0	0	0	0	0	0		0	0	0				
Cells: group O segments, washed 3x in Saline, 1x in LISS															
Antoglobulin Serum: Pfizer 73051															

False-Positive Reactions using LISS

(b). 20 negative sera tested with monospecific antiglobulin sera.

LISS suspended red cells 10 mins. incubation at room temperature
37C for 10 mins.

	RT + 37C + IAT			Anti-IgG	Anti-IgM	Anti-IgA	Anti-C3	Anti-C4
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0 ^s
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0 ^s
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0 ^s
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	2 ⁺
20	0	0	0	0	0	0	0	0 ^s

Cells: Group O segments, washed 3x in saline 1x LISS

Anti-IgG r, 4, 5, 6, (1:128)

Anti-C3: R123+ (1:8)

Anti-IgM Goat 8-6-74(1:10)

Anti-C4: ABC (1:8)

Anti-IgA: Goat 125, 26, 4b, 40, 32, 20, 1-30-75(1:20)

3: FALSE POSITIVE REACTIONS USING LISS

a) 100 sera from unselected hospital patients were tested against a pool of two screening cells (i.e., containing most common blood group antigens). The cells were washed x3 in normal saline and then resuspended to 2% in LISS. Two volumes of these cells were added to two volumes of sera and incubated at 37°C for 10 minutes (incubation time selected from previous experiments) and then washed x4 and tested with commercial (Ortho) antiglobulin sera. If a positive result was found the sera was retested in parallel with albumin suspended red cells and also tested against a panel of phenotyped red cells to identify the specificity of the antibody.

LISS		LISS	
SERA	INDIRECT ANTIGLOBULIN TEST	SERA	INDIRECT ANTIGLOBULIN TEST
1	0	35	0
2	0	36	0
3	0	37	0
4	0	38	3 (Alb IAT 3+)
5	0		Anti-Le ^b
6	1/2 (Alb IAT Negative)	39	0
	Unidentified	40	0
7	0	41	0
8	0	42	0
9	0	43	0
10	0	44	0
11	0	45	0
12	0	46	0
13	0	47	0
14	0	48	0
15	0	49	0
16	0	50	0
17	0	51	0
18	0	52	0
19	0	53	0
20	0	54	0
21	0	55	0
22	0	56	0
23	0	57	0
24	0	58	0
25	0	59	0
26	0	60	0
27	0	61	0
28	0	62	0
29	0	63	0
30	0	64	0
31	0	65	0
32	0	66	0
33	0	67	0
34	3 (Alb IAT 1 1/2).	68	0
	Anti-D		

LISS INDIRECT ANTIGLOBULIN TEST		SERA	LISS INDIRECT ANTIGLOBULIN TEST	
69	0	85	0	
70	0	86	0	
71	3 (Alb IAT Negative) Unidentified	87	0	
72	0	88	0	
73	0	89	0	
74	0	90	0	
75	0	91	0	
76	0	92	0	
77	0	93	0	
78	0	94	0	
79	0	95	0	
80	0	96	0	
81	0	97	0	
82	0	98	0	
83	0	99	0	
84	0	100	0	

b) 21 of the negative sera were further tested by indirect antiglobulin test using anti-IgG, -IgA, -C3 and -C4 at optimal dilutions.

Antiglobulin Sera

	Anti-IgG	Anti-IgA	Anti-C3	Anti-C4
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0

SUMMARY OF RESULTS OF PART 3

When sera from 100 unselected hospital patients were tested four positive results were obtained. One serum contained anti-D and another anti-Le^b; both of these antibodies reacted by "non-LISS" methods. Two of the sera contained antibodies reacting by LISS techniques only but unfortunately insufficient sera was available to identify these antibodies.

No non-specific results were observed when sera from 100 normal blood donors were tested by the LISS method.

The use of powerful monospecific anti-IgG, -IgA, -C3 and -C4 reagents, prepared in our own laboratory, yielded no false positive reactions either.

4. COMPARISON OF SALINE, ALBUMIN AND LISS SENSITIVITY
IN ANTIBODY DETECTION

One hundred antibodies were tested against saline, albumin and low ionic strength solution (LISS) suspended red cells. Fifty of the antibodies were IgM agglutinating antibodies; 24 of the sera contained IgM agglutinating and IgG sensitizing Rh antibodies and 26 reacted only by indirect antiglobulin test.

The 50 agglutinating antibodies were read after immediate centrifugation and incubation at room temperature (24C) or 20C for ten minutes. The antiglobulin reactive antibodies were read for agglutination following:

- a) Immediate centrifugation, then followed by washing x4 and addition of antiglobulin serum.
- b) 37C incubation for optimal incubation period (i.e., 60 minutes for saline and albumin and 10 minutes for LISS) then followed by washing x4 and addition of antiglobulin serum.

4. COMPARISON OF SENSITIVITY IN ANTIBODY DETECTION

Direct Agglutination

1. ABO

Sera from hospital patients tested against A cells or B cells, as appropriate. Read after immediate centrifugation (I.C.) and following incubation at room temperature (24°C) for 10 minutes.

	Saline		Albumin		LISS	
	IC	10 mins	IC	10 mins	IC	10 mins
<u>Group O</u>						
#1	4	4	4	4	4	4
#2	3	3	3	3	2	3
#3	4	4	4	4	4	4
#4	1½	2	1	2	1	1½
#5	4	4	4	4	4	4
#6	4	4	2	3	3	3½
#7	4	4	4	4	4	4
#8	4	4	3	4	4	4
#9	4	4	3	4	4	4
#10	3	4	2½	4	3	4
<u>Group A</u>						
#1	2½	3	1	3	2½	3
#2	4	4	1½	3	3	4
#3	3	3	1	2½	3	3
#4	4	4	2½	3½	4	4
#5	2½	3	1	2½	1½	3
#6	3	3½	2½	3½	3	3½

Direct Agglutination continued

	Saline		Albumin		LISS	
	IC	10 mins	IC	10 mins	IC	10 mins
<u>Group B</u>						
#1	4	4	4	4	4	4
#2	4	4	3	4	4	4
#3	4	4	3½	4	4	4
#4	4	4	4	4	4	4
#5	4	4	3	4	4	4

2. Anti-I (10 Minutes at 24°C)

#1	0	1	0	2	0	2
#2	0	1½	0	2½	0	1
#3	0	1	0	4	0	(1)
#4	0	2½	0	1	0	1
#5	0	1	0	1	0	1
#6	0	2	0	3	0	2

3. Anti-H (10 Minutes at 4°C)

#1	0	1	0	1½	0	1
#2	0	1	0	2	0	1½
#3	0	1½	0	1½	0	1
#4	0	2	0	2	0	2

4. Anti-M (10 Minutes at 20°C)

#1	0	1½	0	1½	0	1½
#2	0	1	0	1	0	1
#3	0	1	0	1	0	1
#4	0	0	0	0	0	(1)
#5	0	0	0	0	0	1

Direct Agglutination

	Saline		Albumin		LISS	
	IC	10 mins	IC	10 mins	IC	10 mins
<u>5. Anti-N</u> (10 Minutes at 20°C)						
#1	0	2½	0	2	0	2
#2	0	2½	0	2½	0	2½
#3	0	3	0	2½	0	2
#4	0	1	0	(1)	0	1
#5	0	3	0	2½	0	2½
<u>6. Anti-P₁</u> (10 Minutes at 20°C)						
#1	0	3	0	3	0	3
#2	0	1	0	1	0	1½
#3	0	1	0	2	0	1½
<u>7. Anti-Le^a</u> (10 Minutes at 20°C)						
#1	0	1	0	2	0	1
#2	0	0	0	1	0	0
#3	0	0	0	2½	0	0
<u>8. Anti-Le^b</u> (10 Minutes at 20°C)						
#1	0	1½	0	1½	0	1
#2	0	½	0	1½	0	½
<u>9. Anti-Rho (D)</u> (10 Minutes at 37°C)						
#1	0	3	0	2½	0	3½

Indirect Antiglobulin Test (IAT)

All tests were read for agglutination following:

- a) Immediate centrifugation, then followed by washing x4 and addition of antiglobulin serum
- b) 37°C incubation for optimal incubation period (i.e., 60 minutes for saline and albumin and 10 minutes for LISS) then followed by washing x4 and addition of antiglobulin serum.

a) Immediate Centrifugation (IC) → Indirect Antiglobulin Test (IAT)

	Saline		Albumin		LISS	
	IC	IAT	IC	IAT	IC	IAT
<hr/>						
<u>Anti-D</u>						
#1	2	4	4	4	0	4
#2	2	3	3	3	2	2
#3	0	(1)	0	(1)	0	(1)
#4	1	3	1	3	0	3
#5	2	3	2	3	1	3
#6	(1)	3	(1)	3	(1)	3½
#7	1	3½	3	3½	(1)	3½
#8	3	4	3	4	3½	4
#9	1½	3½	3	3½	1	3½
#10	½	3	3	3	(1)	3
#11	0	3½	2	3½	(1)	3½
#12	0	4	3	4	0	4

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	IC	IAT	IC	IAT	IC	IAT
<u>Anti-E</u>						
#1	0	1½	0	(1)	0	1½
#2	(1)	1	(1)	2	(1)	2
#3	(1)	2	(1)	2	(1)	2
#4	1½	2	(1)	2	1½	2
#5	1	3	1	2½	1	3½
<u>Anti-C</u>						
#1	(1)	3	(1)	2½	(1)	3½
#2	(1)	(1)	(1)	(1)	(1)	1
<u>Anti-C̄</u>						
#1	(1)	3	2	3	(1)	3½
#2	1½	2	1½	1½	1½	2½
#3	2	3	1	3	2	3½
<u>Anti-ē</u>						
#1	(1)	1½	(1)	1	(1)	2
#2	(1)	1	(1)	1	(1)	1½
<u>Anti-Le^a</u>						
#1	0	0	0	0	0	0
#2	0	0	0	0	0	0
#3	0	0	0	0	0	0
#4	0	0	0	0	0	0
#5	0	0	0	0	0	0

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	IC	IAT	IC	IAT	IC	IAT
<u>Anti-Le^b</u>						
#1	0	0	0	0	0	0
#2	0	0	0	0	0	0
<u>Anti-S</u>						
#1	0	(1)	0	(1)	0	1
#2	0	0	0	0	0	(1)
<u>Anti-s̄</u>						
#1	0	(1)	0	(1)	0	2
#2	0	1	0	1½	0	2
<u>Anti-Lu^a</u>						
#1	0	0	0	0	0	0
#2	0	0	0	0	0	0
<u>Anti-Lu^b</u>						
#1	0	(1)	0	(1)	0	(1)
#2	0	½	0	½	0	½
<u>Anti-K</u>						
#1	0	1½	0	1½	0	2
#2	0	(1)	0	(1)	0	(1)
<u>Anti-K̄</u>						
#1	0	2½	0	2½	0	3
#2	0	3	0	3	0	3

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	IC	IAT	IC	IAT	IC	IAT
<u>Anti-Fy^a</u>						
#1	0	(1)	0	(1)	0	1
#2	0	0	0	0	0	0
<u>Anti-Fy^b</u>						
#1	0	(1)	0	(1)	0	1½
#2	0	1	0	1	0	1½
<u>Anti-Jk^a</u>						
#1	0	1½	0	(1)	0	2
#2	0	1	0	(1)	0	2½
<u>Anti-Jk^b</u>						
#1	0	0	0	0	0	0
#2	0	0	0	0	0	0

b) Optimal Incubation Period → IAT

	Saline		Albumin		LISS	
	1 hr	IAT	1 hr	IAT	10 mins	IAT
<u>Anti-D</u>						
#1	0	4	(1)	4	0	4
#2	(1)	3½	3	3½	0	3½
#3	0	(1)	0	(1)	0	(1)
#4	1	3½	1	3½	1	3½
#5	(1)	4	(1)	4	(1)	4
#6	3	3½	3	3½	3½	3½

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	1 hr	IAT	1 hr	IAT	10 mins	IAT
<u>Anti-D (cont.)</u>						
#7	0	4	1	4	0	4
#8	0	4	2	4	0	4
#9	1½	4	3	4	1	4
#10	(1)	3	2½	3	0	3½
#11	0	3½	3	3½	0	3½
#12	0	4	1	4	0	4
<u>Anti-E</u>						
#1	1½	2	2	2½	2½	3
#2	1½	2½	2	3	2½	3
#3	2½	3½	2½	3½	2½	3½
#4	1½	4	1	4	1½	4
#5	2½	3	2½	3	2½	4
<u>Anti-C</u>						
#1	1½	3	1½	3	1½	3
#2	1	1½	1	1½	1	2
<u>Anti-Ā</u>						
#1	1	4	2	4	1½	4
#2	2	3½	2½	3	2½	3½
#3	3	3	2	3	3	3½
<u>Anti-ē</u>						
#1	1½	2½	1	2½	1½	2½
#2	1½	1½	1	1½	1½	2

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	1 hr	IAT	1 hr	IAT	10 mins	IAT
<hr/>						
<u>Anti-Le^a</u>						
#1	0	0	0	(1)	0	0
#2	0	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$
#3	0	1	0	1	0	1
#4	0	$\frac{1}{2}$	0	1	0	(1)
#5	0	$\frac{1}{2}$	0	(1)	0	1
<u>Anti-Le^b</u>						
#1	0	0	0	0	0	0
#2	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$
<u>Anti-S</u>						
#1	0	1	0	1	0	$1\frac{1}{2}$
#2	0	(1)	0	(1)	0	1
<u>Anti-s</u>						
#1	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	$2\frac{1}{2}$
#2	0	$1\frac{1}{2}$	0	2	0	2
<u>Anti-Lu^a</u>						
#1	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$
#2	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$
<u>Anti-Lu^b</u>						
#1	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$
#2	0	1	0	1	0	$1\frac{1}{2}$
<u>Anti-K</u>						
#1	0	1	0	1	0	$1\frac{1}{2}$
#2	0	(1)	0	(1)	0	1

Indirect Antiglobulin Test (IAT) continued

	Saline		Albumin		LISS	
	1 hr	IAT	1 hr	IAT	10 mins	IAT
<hr/>						
<u>Anti-K</u>						
#1	0	2½	0	2½	0	3
#2	0	3½	0	3½	0	3½
<u>Anti-Fy^a</u>						
#1	0	1½	0	1½	0	1½
#2	0	(1)	0	(1)	0	(1)
<u>Anti-Fy^b</u>						
#1	0	1½	0	1½	0	2
#2	0	1	0	2	0	1½
<u>Anti-Jk^a</u>						
#1	0	2½	0	2½	0	3
#2	0	2½	0	2½	0	2½
<u>Anti-Jk^b</u>						
#1	0	(1)	0	(1)	0	(1)
#2	0	2	0	2	0	2

SUMMARY OF RESULTS OF PART 4

One hundred antibodies were tested against saline, albumin and low ionic strength solution (LISS) suspended red cells. Fifty of the antibodies were IgM agglutinating antibodies; 24 of the sera contained IgM agglutinating and IgG sensitizing Rh antibodies and 26 reacted only by indirect antiglobulin test.

The 50 agglutinating antibodies were read after immediate centrifugation and incubation at room temperature (24°C) or 20°C for ten minutes. All of the antibodies, except two reacted in saline, albumin or LISS. The two exceptions were anti-M that were only detected in LISS.

The Rh antibodies all reacted by indirect antiglobulin test in saline, albumin or LISS but some of the IgM agglutination reactions were only detectable by immediate centrifugation (probably because the powerful IgG antibodies present blocked available Rh antigenic sites on incubation). One such reaction was undetectable in LISS on immediate centrifugation but detectable in saline or albumin. It should be noted that this same serum reacted well when the immediate centrifugation test in LISS was tested with anti-globulin serum.

Of the other 26 antiglobulin reactive antibodies, one anti-Le^a was not detectable in albumin whereas one other anti-Le^a was detectable in LISS and albumin but not saline. All other antibodies were detectable in all three systems. Except for the anti-Le^a mentioned, all antibodies reacted equal or better in the LISS system (e.g., 2/2 anti-S, 2/2 anti-s, 2/2 anti-K, 1/2 anti-Fy^a, 2/2 anti-Fy^b and 1/2 anti-Jk^a tested reacted better in LISS than saline and albumin).

It should once again be noted that the LISS incubation time is only ten minutes compared to one hour for saline and albumin.

5. Specificity and Sensitivity Under Routine Conditions

1000 sera from unselected hospital patients were tested against red cells from donor units. Conventional methods using saline-suspended red cells with and without the addition of 30% albumin were compared with duplicate tests using the same red cells suspended in LISS. Optimal incubation times were used. All tests were incubated at room temperature and then 37C, read for agglutination at both temperatures and sensitization detected by the antiglobulin test. All negative reactions were checked microscopically. Any positive reactions were investigated to determine antibody specificity.

Pooled Group O segment from PMC blood bank.

Sal cells: washed 3x in saline 5%

LISS cells: washed 3x in saline 1x in LISS 2%

Sera (St. Joseph)

	15'		30'		IAT (imm)		5'		15'	10'	IAT	IAT
	RT (24C)		37C				IAT		RT	37C		
	SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4 NUENEZ	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

		RT	15' (24C)	30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
25		0	0	0	0	0	0	0	0	0	0	0	0
26		0	0	0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0
30		0	0	0	0	0	0	0	0	0	0	0	0
31	MURTER DREW, 7-27-77	0	1	0	1 1/2	4	3	4	3	0	0	3	3
32		0	0	0	0	0	0	0	0	0	0	0	0
33		0	0	0	0	0	0	0	0	0	0	0	0
34	WEDDINGTON, PATRICIA	0	1 1/2	0	0	0	0	0	0	0	0	0	0
35		0	0	0	0	0	0	0	0	0	0	0	0
36		0	0	0	0	0	0	0	0	0	0	0	0
37		0	0	0	0	0	0	0	0	0	0	0	0
38		0	0	0	0	0	0	0	0	0	0	0	0
39		0	0	0	0	0	0	0	0	0	0	0	0
40		0	0	0	0	0	0	0	0	0	0	0	0
41		0	0	0	0	0	0	0	0	0	0	0	0
42		0	0	0	0	0	0	0	0	0	0	0	0
43		0	0	0	0	0	0	0	0	0	0	0	0
44		0	0	0	0	0	0	0	0	0	0	0	0
45		0	0	0	0	0	0	0	0	0	0	0	0
46		0	0	0	0	0	0	0	0	0	0	0	0
47		0	0	0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

		15' (24C)		30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
49		0	0	0	0	0	0	0	0	0	0	0	0
50		0	0	0	0	0	0	0	0	0	0	0	0
51		0	0	0	0	0	0	0	0	0	0	0	0
52		0	0	0	0	0	0	0	0	0	0	0	0
53		0	0	0	0	0	0	0	0	0	0	0	0
54		0	0	0	0	0	0	0	0	0	0	0	0
55		0	0	0	0	0	0	0	0	0	0	0	0
56	MILD, EARLY NO DATE	0	0	0	0	0	0	0	0	(1)	0	0	0
57		0	0	0	0	0	0	0	0 ^s	0	0	0	0
58		0	0	0	0	0	0	0	0	0	0	0	0
59		0	0	0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0	0	0
61		0	0	0	0	0	0	0	0	0	0	0	0
62		0	0	0	0	0	0	0	0	0	0	0	0
63		0	0	0	0	0	0	0	0	0	0	0	0
64		0	0	0	0	0	0	0	0	0	0	0	0
65		0	0	0	0	0	0	0	0	0	0	0	0
66		0	0	0	0	0	0	0	0	0	0	0	0
67		0	0	0	0	0	0	0	0	0	0	0	0
68		0	0	0	0	0	0	0	0	0	0	0	0
69		0	0	0	0	0	0	0	0	0	0	0	0
70		0	0	0	0	0	0	0	0	0	0	0	0
71		0	0	0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

		15' (24C)		30' 37C		IAT (imm)		5' IAT	15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS	LISS
73		0	0	0	0	0	0	0 0	0	0	0	0
74		0	0	0	0	0	0	0 0	0	0	0	0
75		0	0	0	0	0	0	0 0	0	0	0	0
76	BACKMAN, ERIKA 12-28-77	0	0	0	0	$\frac{1}{2}$	(1)	($\frac{1}{2}$) $\frac{1}{2}$	0	0	(1)	0 ^s
77		0	0	0	0	0	0	0 0	0	0	0	0
78		0	0	0	0	0	0	0 0	0	0	0	0
79		0	0	0	0	0	0	0 0	0	0	0	0
80		0	0	0	0	0	0	0 0	0	0	0	0
81		0	0	0	0	0	0	0 0	0	0	0	0
82		0	0	0	0	0	0	0 0	0	0	0	0
83		0	0	0	0	0	0	0 0	0	0	0	0
84		0	0	0	0	0	0	0 0	0	0	0 ^s	0
85		0	0	0	0	0	0	0 0	0	0	0	0
86		0	0	0	0	0	0	0 0	0	0	0	0
87		0	0	0	0	0	0	0 0	0	0	0	0
88		0	0	0	0	0	0	0 0	0	0	0 ^s	0
89		0	0	0	0	0	0	0 0	0	0	0	0
90		0	0	0	0	0	0	0 0	0	0	0	0
91		0	0	0	0	0	0	0 0	0	0	0	0
92	BACKMAN	0	0	0	0	0	$\frac{1}{2}$	0 ($\frac{1}{2}$)	0	0	(1)	(1)
93		0	0	0	0	0	0	0 0	0	0	0 ^s	0
94		0	0	0	0	0	0	0 0	0	0	0	0
95		0	0	0	0	0	0	0 0	0	0	0	0
96	KATSUJI	0	0	0	0	0	0	0 0	0	0	$\frac{1}{2}$	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

	RT	15' (24C)	30' 37C		IAT (imm)		5' IAT	15' RT	10' 37C	IAT	IAT (5C)
	SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS	LISS
97	0	0	0	0	0	0	0 0	0	0	0	0
98	0	0	0	0	0	0	0 0	0	0	0	0
99	0	0	0	0	0	0	0 0	0	0	0	0
100	0	0	0	0	0	0	0 0	0	0	0	0
101	0	0	0	0	0	0	0 0	0	0	0	0
102	0	0	0	0	0	0	0 0	0	0	0	0
103	0	0	0	0	0	0	0 0	0	0	0	0
104	0	0	0	0	0	0	0 0	0	0	0	0
105	0	0	0	0	0	0	0 0	0	0	0	0
106	OLSON, LAURA	0	0	0	0	0	0 0	0	0	1/2	0
107		0	0	0	0	0	0 0	0	0	0	0
108		0	0	0	0	0	0 0	0	0	0	0
109		0	0	0	0	0	0 0	0	0	0	0
110		0	0	0	0	0	0 0	0	0	0	0
111		0	0	0	0	0	0 0	0	0	0	0
112		0	0	0	0	0	0 0	0	0	0	0
113		0	0	0	0	0	0 0	0	0	0	0
114		0	0	0	0	0	0 0	0	0	0	0
115		0	0	0	0	0	0 0	0	0	0	0
116		0	0	0	0	0	0 0	0	0	0	0
117		0	0	0	0	0	0 0	0	0	0	0
118		0	0	0	0	0	0 0	0	0	0	0
119		0	0	0	0	0	0 0	0	0	0	0
120		0	0	0	0	0	0 0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

		RT	15' (24C)	30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
121		0	0	0	0	0	0	0	0	0	0	0	0
122		0	0	0	0	0	0	0	0	0	0	0	0
123		0	0	0	0	0	0	0	0	0	0	0	0
124		0	0	0	0	0	0	0	0	0	0	0	0
125		0	0	0	0	0	0	0	0	0	0	0	0
126		0	0	0	0	0	0	0	0	0	0	0	0
127		0	0	0	0	0	0	0	0	0	0	0	0
128		0	0	0	0	0	0	0	0	0	0	0	0
129		0	0	0	0	0	0	0	0	0	0	0	0
130		0	0	0	0	0	0	0	0	0	0	0	0
131		0	0	0	0	0	0	0	0	0	0	0	0
132		0	0	0	0	0	0	0	0	0	0	0	0
133		0	0	0	0	0	0	0	0	0	0	0	0
134		0	0	0	0	0	0	0	0	0	0	0	0
135		0	0	0	0	0	0	0	0	0	0	0	0
136		0	0	0	0	0	0	0	0	0	0	0	0
137		0	0	0	0	0	0	0	0	0	0	0	0
138		0	0	0	0	0	0	0	0	0	0	0	0
139		0	0	0	0	0	0	0	0	0	0	0	0
140		0	0	0	0	0	0	0	0	0	0	0	0
141		0	0	0	0	0	0	0	0	0	0	0	0
142		0	0	0	0	0	0	0	0	0	0	0	0
143		0	0	0	0	0	0	0	0	0	0	0	0
144		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: ST. JOSEPH'S

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
145		0	0	0	0	0	0	0	0	0	0	0	0
146		0	0	0	0	0	0	0	0	0	0	0	0
147		0	0	0	0	0	0	0	0	0	0	0	0
148	1-2-78 LE -CHENKO	0	1	0	0	0	0	0	0	0	0	0	0
149		0	0	0	0	0	0	0	0	0	0	0	0
150		0	0	0	0	0	0	0	0	0	0	0	0
151	MCINTOSH, ALICE 1-2-78	0	0	0	0	0	0	0	0	0	0	(1)	$\frac{1}{2}$
152		0	0	0	0	0	0	0	0	0	0	0	0
153		0	0	0	0	0	0	0	0	0	0	0	0
154		0	0	0	0	0	0	0	0	0	0	0	0
155		0	0	0	0	0	0	0	0	0	0	0	0
156		0	0	0	0	0	0	0	0	0	0	0	0
157		0	0	0	0	0	0	0	0	0	0	0	0
158		0	0	0	0	0	0	0	0	0	0	0	0
159		0	0	0	0	0	0	0	0	0	0	0	0
160		0	0	0	0	0	0	0	0	0	0	0	0
161		0	0	0	0	0	0	0	0	0	0	0	0
162		0	0	0	0	0	0	0	0	0	0	0	0
163		0	0	0	0	0	0	0	0	0	0	0	0
164		0	0	0	0	0	0	0	0	0	0	0	0
165		0	0	0	0	0	0	0	0	0	0	0	0
166		0	0	0	0	0	0	0	0	0	0	0	0
167		0	0	0	0	0	0	0	0	0	0	0	0
168		0	0	0	0	0	0	0	0	0	0	0	0

R.T. = 24C

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: PMC

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
25		0	0	0	0	0	0	0	0	0	0	0	0
26		0	0	0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0
30		0	0	0	0	0	0	0	0	0	0	0	0
31		0	0	0	0	0	0	0	0	0	0	0	0
32		0	0	0	0	0	0	0	0	0	0	0	0
33		0	0	0	0	0	0 ^s	0	0 ^s	0	0	0 ^s	0 ^s
34		0	0	0	0	0	0	0	0	0	0	0	0
35		0	0	0	0	0	0	0	0	0	0	0	0
36	URRAUS, S.	0	3	0	3	3	3	0	0	0	0	4	4
37		0	0	0	0	0	0	0	0	0	0	0	0
38	MARK, F 1-9-78	0	0	0	0	0	0	0	0	1	0	0 ^s	0 ^s
39		0	0	0	0	0	0	0	0	0	0	0	0
40		0	0	0	0	0	0	0	0	0	0	0	0
41		0	0	0	0	0	0	0	0	0	0	0	0
42		0	0	0	0	0	0	0	0	0	0	0	0
43		0	0	0	0	0	0	0	0	0	0	0	0
44		0	0	0	0	0	0	0	0	0	0	0	0
45		0	0	0	0	0	0	0	0	0	0	0	0
46		0	0	0	0	0	0	0	0	0	0	0	0
47		0	0	0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: PMC

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
49	LEONARD, R. 1-10-78	0	0	0	0	0	0	0	0	(1)	0	0	0
50		0	0	0	0	0	0	0	0	0	0	0	0
51	HALE, W. 1-10-78	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	0
52		0	0	0	0	0	0	0	0	0	0	0	0
53		0	0	0	0	0	0	0	0	0	0	0	0
54		0	0	0	0	0	0	0	0	0	0	0	0
55		0	0	0	0	0	0	0	0	0	0	0	0
56		0	0	0	0	0	0	0	0	0	0	0	0
57		0	0	0	0	0	0	0	0	0	0	0	0
58		0	0	0	0	0	0	0	0	0	0	0	0
59	ELLIS, J. 1-10-78	0	0	0	0	(1)	(1)	(1)	(1)	0	0	(1)	(1)
60		0	0	0	0	0	0	0	0	0	0	0	0
61		0	0	0	0	0	0	0	0	0	0	0	0
62		0	0	0	0	0	0	0	0	0	0	0	0
63		0	0	0	0	0	0	0	0	0	0	0	0
64		0	0	0	0	0	0	0	0	0	0	0	0
65		0	0	0	0	0	0	0	0	0	0	0	0
66		0	0	0	0	0	0	0	0	0	0	0	0
67		0	0	0	0	0	0	0	0	0	0	0	0
68		0	0	0	0	0	0	0	0	0	0	0	0
69		0	0	0	0	0	0	0	0	0	0	0	0
70		0	0	0	0	0	0	0	0	0	0	0	0
71		0	0	0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: *PMC*

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
73	<i>YIM, G.</i> <i>1-11-78</i>	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	0 ^s
74		0	0	0	0	0	0	0	0	0	0	0	0
75	<i>SKRAGG, P.</i> <i>1-12-78</i>	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	0 ^s
76		0	0	0	0	0	0	0	0	0	0	0	0
77		0	0	0	0	0	0	0	0	0	0	0	0
78		0	0	0	0	0	0	0	0	0	0	0	0
79		0	0	0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0	0	0
81		0	0	0	0	0	0	0	0	0	0	0	0
82		0	0	0	0	0	0	0	0	0	0	0	0
83		0	0	0	0	0	0	0	0	0	0	0	0
84		0	0	0	0	0	0	0	0	0	0	0	0
85	<i>REGGIARDO, ELIZABETH</i> <i>1-11-78</i>	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	0
86		0	0	0	0	0	0	0	0	0	0	0	0
87		0	0	0	0	0	0	0	0	0	0	0	0
88		0	0	0	0	0	0	0	0	0	0	0	0
89		0	0	0	0	0	0	0	0	0	0	0	0
90	<i>GALAN, SIMENA</i> <i>1-12-78</i>	0	0	0	0	0	0	0	0	0	0	(1)	(1)
91		0	0	0	0	0	0	0	0	0	0	0	0
92		0	0	0	0	0	0	0	0	0	0	0	0
93		0	0	0	0	0	0	0	0	0	0	0	0
94		0	0	0	0	0	0	0	0	0	0	0	0
95		0	0	0	0	0	0	0	0	0	0	0	0
96		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: PMC

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT(imm)		5' IAT	15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS	LISS
97		0	0	0	0	0	0	0 0	0	0	0	0
98		0	0	0	0	0	0	0 0	0	0	0	0
99		0	0	0	0	0	0	0 0	0	0	0	0
100		0	0	0	0	0	0	0 0	0	0	0	0
101		0	0	0	0	0	0	0 0	0	0	0	0
102		0	0	0	0	0	0	0 0	0	0	0	0
103		0	0	0	0	0	0	0 0	0	0	0	0
104		0	0	0	0	0	0	0 0	0	0	0	0
105		0	0	0	0	0	0	0 0	0	0	0	0
106		0	0	0	0	0	0	0 0	0	0	0	0
107		0	0	0	0	0	0	0 0	0	0	0	0
108		0	0	0	0	0	0	0 0	0	0	0	0
109		0	0	0	0	0	0	0 0	0	0	0	0
110		0	0	0	0	0	0	0 0	0	0	0	0
111		0	0	0	0	0	0	0 0	0	0	0	0
112		0	0	0	0	0	0	0 0	0	0	0	0
113		0	0	0	0	0	0	0 0	0	0	0	0
114		0	0	0	0	0	0	0 0	0	0	0	0
115		0	0	0	0	0	0	0 0	0	0	0	0
116		0	0	0	0	0	0	0 0	0	0	0	0
117		0	0	0	0	0	0	0 0	0	0	0	0
118		0	0	0	0	0	0	0 0	0	0	0	0
119		0	0	0	0	0	0	0 0	0	0	0	0
120		0	0	0	0	0	0	0 0	0	0	0	0

Sal cells: washed 3x in saline

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LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: PMC

R.T. = 24C

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Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: PMC

R.T. = 24C

		RT	15' (24C)	30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
145		0	0	0	0	0	0	0	0	0	0	0	0
146		0	0	0	0	0	0	0	0	0	0	0	
147	LEONARD RUSSELL 1-16-78	0	0	0	0	0	0	0	0	2	0	0	0
148		0	0	0	0	0	0	0	0	0	0	0	0
149		0	0	0	0	0	0	0	0	0	0	0	0
150		0	0	0	0	0	0	0	0	0	0	0	0
151		0	0	0	0	0	0	0	0	0	0	0	0
152		0	0	0	0	0	0	0	0	0	0	0	0
153		0	0	0	0	0	0	0	0	0	0	0	0
154		0	0	0	0	0	0	0	0	0	0	0	0
155		0	0	0	0	0	0	0	0	0	0	0	0
156		0	0	0	0	0	0	0	0	0	0	0	0
157		0	0	0	0	0	0	0	0	0	0	0	0
158		0	0	0	0	0	0	0	0	0	0	0	0
159		0	0	0	0	0	0	0	0	0	0	0	0
160		0	0	0	0	0	0	0	0	0	0	0	0
161		0	0	0	0	0	0	0	0	0	0	0	0
162		0	0	0	0	0	0	0	0	0	0	0	0
163		0	0	0	0	0	0	0	0	0	0	0	0
164		0	0	0	0	0	0	0	0	0	0	0	0
165		0	0	0	0	0	0	0	0	0	0	0	0
166		0	0	0	0	0	0	0	0	0	0	0	0
167		0	0	0	0	0	0	0	0	0	0	0	0
168		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: STANFORD

R.T. = 24C

	RT	15' (24C)	30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
	SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	1½	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0

R.T. = 24C

		^{15'} RT (24C)		^{30'} 37C		IAT(inn)		^{5'} IAT		^{15'} RT	^{10'} 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS LISS		
1		O	O	O	O	O	O	O O	O	O	O O		
2		O	O	O	O	O	O	O O	O	O	O O		
3		O	O	O	O	O	O	O O	O	O	O O		
4		O	O	O	O	O	O	O O	O	O	O O		
5		O	O	O	O	O	O	O O	O	O	O O		
6		O	O	O	O	O	O	O O	O	O	O O		
7		O	O	O	O	O	O	O O	O	O	O O		
8		O	O	O	O	O	O	O O	O	O	O O		
9		O	O	O	O	O	O	O O	O	O	O O		
10		O	O	O	O	O	O	O O	O	O	O O		
11		O	O	O	O	O	O	O O	O	O	O O		
12		O	O	O	O	O	O	O O	O	O	O O		
13		O	O	O	O	O	O	O O	O	O	O O		
14		O	O	O	O	O	O	O O	O	O	O O		
15		O	O	O	O	O	O	O O	O	O	O O		
16		O	O	O	O	O	O	O O	O	O	O O		
17		O	O	O	O	O	O	O O	O	O	O O		
18		O	O	O	O	O	O	O O	O	O	O O		
19		O	O	O	O	O	O	O O	O	O	O O		
20		O	O	O	O	O	O	O O	O	O	O O		
21		O	O	O	O	O	O	O O	O	O	O O		
22		O	O	O	O	O	O	O O	O	O	O O		
23		O	O	O	O	O	O	O O	O	O	O O		
24		O	O	O	O	O	O	O O	O	O	O O		

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Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: JHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
25		0	0	0	0	0	0	0	0	0	0	0	0
26		0	0	0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0
30		0	0	0	0	0	0	0	0	0	0	0	0
31		0	0	0	0	0	0	0	0	0	0	0	0
32		0	0	0	0	0	0	0	0	0	0	0	0
33		0	0	0	0	0	0	0	0	0	0	0	0
34		0	0	0	0	0	0	0	0	0	0	0	0
35		0	0	0	0	0	0	0	0	0	0	0	0
36		0	0	0	0	0	0	0	0	0	0	0	0
37		0	0	0	0	0	0	0	0	0	0	0	0
38		0	0	0	0	0	0	0	0	0	0	0	0
39		0	0	0	0	0	0	0	0	0	0	0	0
40		0	0	0	0	0	0	0	0	0	0	0	0
41	R-153817 0580-R	1	0	0	0	0 ^s	0	0 ^s	0	0	0	0 ^s	0 ^s
42		0	0	0	0	0	0	0	0	0	0	0	0
43		0	0	0	0	0	0	0	0	0	0	0	0
44		0	0	0	0	0	0	0	0	0	0	0	0
45		0	0	0	0	0	0	0	0	0	0	0	0
46		0	0	0	0	0	0	0	0	0	0	0	0
47		0	0	0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT	15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS	LISS
49		0	0	0	0	0	0	0 0	0	0	0	0
50		0	0	0	0	0	0	0 0	0	0	0	0
51		0	6	0	0	0	0	0 0	0	0	0	0
52		0	0	0	0	0	0	0 0	0	0	0	0
53		0	0	0	0	0	0	0 0	0	0	0	0
54		0	0	0	0	0	0	0 0	0	0	0	0
55		0	0	0	0	0	0	0 0	0	0	0	0
56		0	0	0	0	0 ^s	0 ^s	0 ^s 0 ^s	0	0	0 ^s	0 ^s
57		0	0	0	0	0	0	0 0	0	0	0	0
58		0	0	0	0	0	0	0 0	0	0	0	0
59		0	0	0	0	0	0	0 0	0	0	0	0
60		0	0	0	0	0	0	0 0	0	0	0	0
61		0	0	0	0	0	0	0 0	0	0	0	0
62	J007252- OGIE	0	0	0	0	0	0	0 0	0	0	1/2	(1/2)
63		0	0	0	0	0	0	0 0	0	0	0	0
64		0	0	0	0	0	0	0 0	0	0	0	0
65		0	0	0	0	0	0	0 0	0	0	0	0
66		0	0	0	0	0	0	0 0	0	0	0	0
67		0	0	0	0	0	0	0 0	0	0	0	0
68		0	0	0	0	0	0	0 0	0	0	0	0
69		0	0	0	0	0	0	0 0	0	0	0	0
70		0	0	0	0	0	0	0 0	0	0	0	0
71		0	0	0	0	0 ^s	0	0 ^s 0	0	0	0 ^s	0
72		0	0	0	0	0	0	0 0	0	0	0	0

R.T. = 24C

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT	15' (24C)	30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
97		0	0	0	0	0	0	0	0	0	0	0	0
98		0	0	0	0	0	0	0	0	1	0	(1)	(1)
99		0	0	0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0	0	0
101	R-049916 LAVE	0	0	0	0	0	0	0	0	0	0	$\frac{1}{2}$	0 ^s
102		0	0	0	0	0	0	0	0	0	0	0	0
103		0	0	0	0	0	0	0	0	0	0	0	0
104		0	0	0	0	0	0	0	0	0	0	0	0
105		0	0	0	0	0	0	0	0	0	0	0	0
106		0	0	0	0	0	0	0	0	0	0	0	0
107		0	0	0	0	0	0	0	0	0	0	0	0
108		0	0	0	0	0	0	0	0	0	0	0	0
109		0	0	0	0	0	0	0	0	0	0	0	0
110		0	0	0	0	0	0	0	0	0	0	0	0
111		0	0	0	0	0	0	0	0	0	0	0	0
112		0	0	0	0	0	0	0	0	0	0	0	0
113		0	0	0	0	0	0	0	0	0	0	0	0
114		0	0	0	0	0	0	0	0	0	0	0	0
115		0	0	0	0	0	0	0	0	0	0	0	0
116		0	0	0	0	0	0	0	0	0	0	0	0
117		0	0	0	0	0	0	0	0	0	0	0	0
118		0	0	0	0	0	0	0	0	0	0	0	0
119	R-037333 MINN	(1)	(1)	0	0	0	0	0	0	0	0	0	0
120	V-088054 CROE	(1)	0	0	0	0	0	0	0	(1)	0	$\frac{1}{2}$	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		15'		30'		IAT (imm)		5'		15'		10'		IAT		IAT	
		RT	(24C)	37C				IAT		RT		37C				(5C)	
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS				
121		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
122		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
123		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
124		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
125		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
126	M-091173 KOPE	0	0	0	0	0	0	0	0	0	0	0	1/2	(1)			
127		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
128		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
129		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
130		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
131		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
132		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
133		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
134		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
135		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
136		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
137		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
138		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
139		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
140		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
141		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
142		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
143		0	0	0	0	0	0	0	0	0	0	0	0	0	0		
144		0	0	0	0	0	0	0	0	0	0	0	0	0	0		

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Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

[illegible]

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

	RT	15' (24C)	30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
	SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
169	0	0	0	0	0	0	0	0	0	0	0	0
170	0	0	0	0	0	0	0	0	0	0	0	0
171	0	0	0	0	0	0	0	0	0	0	0	0
172	0	0	0	0	0	0	0	0	0	0	0	0
173	0	0	0	0	0	0	0	0	0	0	0	0
174	0	0	0	0	0	0	0	0	0	0	0	0
175	0	0	0	0	0	0	0	0	0	0	0	0
176	0	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	0	0	0	0	0	0	0
178	0	0	0	0	0	0	0	0	0	0	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0
183	0	0	0	0	0	0	0	0	0	0	0	0
184	0	0	0	0	0	0	0	0	0	0	0	0
185	0	0	0	0	0	0	0	0	0	0	0	0
186	0	0	0	0	0	0	0	0	0	0	0	0
187	0	0	0	0	0	0	0	0	0	0	0	0
188	0	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0	0	0	0
190	0	0	0	0	0	0	0	0	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0

SERA from: IHR

$$R.T. = 24^{\circ}C$$

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Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

	RT	15' (24C)	30' 37C		IAT(imm)		5' IAT	15' RT	10' 37C	IAT	IAT (5C)
	SAL	ALB	SAL	ALB	SAL	ALB	SAL ALB	LISS	LISS	LISS	LISS
217	0	0	0	0	0	0	0 0	0	0	0	0
218	0	0	0	0	0	0	0 0	0	0	0	0
219	0	0	0	0	0	0	0 0	0	0	0	0
220	0	0	0	0	0	0	0 0	0	0	0	0
221	0	0	0	0	0	0	0 0	0	0	0	0
222	0	0	0	0	0	0	0 0	0	0	0	0
223	0	0	0	0	0	0	0 0	0	0	0	0
224	0	0	0	0	0	0	0 0	0	0	0	0
225	0	0	0	0	0	0	0 0	0	0	0	0
226	0	0	0	0	0	0	0 0	0	0	0	0
227	0	0	0	0	0	0	0 0	0	0	0	0
228	0	0	0	0	0	0	0 0	0	0	0	0
229	0	0	0	0	0	0	0 0	0	0	0	0
230	0	0	0	0	0	0	0 0	0	0	0	0
231	0	0	0	0	0	0	0 0	0	0	0	0
232	0	0	0	0	0	0	0 0	0	0	0	0
233	0	0	0	0	0	0	0 0	0	0	0	0
234	0	0	0	0	0	0	0 0	0	0	0	0
235	0	0	0	0	0	0	0 0	0	0	0	0
236	0	0	0	0	0	0	0 0	0	0	0	0
237	0	0	0	0	0	0	0 0	0	0	0	0
238	0	0	0	0	0	0	0 0	0	0	0	0
239	0	0	0	0	0	0	0 0	0	0	0	0
240	0	0	0	0	0	0	0 0	0	0	0	0

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
241		0	0	0	0	0	0	0	0	0	0	0	0
242		0	0	0	0	0	0	0	0	0	0	0	0
243		0	0	0	0	0	0	0	0	0	0	0	0
244		0	0	0	0	0	0	0	0	0	0	0	0
245		0	0	0	0	0	0	0	0	0	0	0	0
246		0	0	0	0	0	0	0	0	0	0	0	0
247		0	0	0	0	0	0	0	0	0	0	0	0
248		0	0	0	0	0	0	0	0	0	0	0	0
249		0	0	0	0	0	0	0	0	0	0	0	0
250		0	0	0	0	0	0	0	0	0	0	0	0
251		0	0	0	0	0	0	0	0	0	0	0	0
252		0	0	0	0	0	0	0	0	0	0	0	0
253		0	0	0	0	0	0	0	0	0	0	0	0
254		0	0	0	0	0	0	0	0	0	0	0	0
255		0	0	0	0	0	0	0	0	0	0	0	0
256		0	0	0	0	0	0	0	0	0	0	0	0
257		0	0	0	0	0	0	0	0	0	0	0	0
258		0	0	0	0	0	0	0	0	0	0	0	0
259		0	0	0	0	0	0	0	0	0	0	0	0
260		0	0	0	0	0	0	0	0	0	0	0	0
261		0	0	0	0	0	0	0	0	0	0	0	0
262		0	0	0	0	0	0	0	0	0	0	0	0
263		0	0	0	0	0	0	0	0	0	0	0	0
264		0	0	0	0	0	0	0	0	0	0	0	0
265		0	0	0	0	0	0	0	0	0	0	0	0

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Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24°C

[illegible]

R.T. = 24C

[illegible]

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		15' (24C)		30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
362		0	0	0	0	0	0	0	0	0	0	0	0
363		0	0	0	0	0	0	0	0	0	0	0	0
364		0	0	0	0	0	0	0	0	0	0	0	0
365		0	0	0	0	0	0	0	0	0	0	0	0
366		0	0	0	0	0	0	0	0	0	0	0	0
367		0	0	0	0	0	0	0	0	0	0	0	0
368		0	0	0	0	0	0	0	0	0	0	0	0
369		0	0	0	0	0	0	0	0	0	0	0	0
370		0	0	0	0	0	0	0	0	0	0	0	0
371		0	0	0	0	0	0	0	0	0	0	0	0
372	JILL	0	(1)	0	0	0 ^s	0 ^s	0 ^s	0 ^s	0	0	0 ^s	0
373		0	0	0	0	0	0	0	0	0	0	0	0
374		0	0	0	0	0	0	0	0	0	0	0	0
375		0	0	0	0	0	0	0	0	0	0	0	0
376		0	0	0	0	0	0	0	0	0	0	0	0
377	STEW	(1)	1	0	0	($\frac{1}{2}$)	$\frac{1}{2}$	($\frac{1}{2}$)($\frac{1}{2}$)	(1)	0	$\frac{1}{2}$	($\frac{1}{2}$)	
378		0	0	0	0	0	0	0	0	0	0	0	0
379		0	0	0	0	0	0	0	0	0	0	0	0
380		0	0	0	0	0	0	0	0	0	0	0	0
381		0	0	0	0	0	0	0	0	0	0	0	0
382		0	0	0	0	0	0	0	0	0	0	0	0
383		0	0	0	0	0	0	0	0	0	0	0	0
384		0	0	0	0	0	0	0	0	0	0	0	0
385		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
386		0	0	0	0	0	0	0	0	0	0	0	0
387		0	0	0	0	0	0	0	0	0	0	0	0
388		0	0	0	0	0	0	0	0	0	0	0	0
389		0	0	0	0	0	0	0	0	0	0	0	0
390		0	0	0	0	0	0	0	0	0	0	0	0
391		0	0	0	0	0	0	0	0	0	0	0	0
392		0	0	0	0	0	0	0	0	0	0	0	0
393		0	0	0	0	0	0	0	0	0	0	0	0
394		0	0	0	0	0	0	0	0	0	0	0	0
395		0	0	0	0	0	0	0	0	0	0	0	0
396		0	0	0	0	0	0	0	0	0	0	0	0
397		0	0	0	0	0	0	0	0	0	0	0	0
398		0	0	0	0	0	0	0	0	0	0	0	0
399		0	0	0	0	0	0	0	0	0	0	0	0
400		0	0	0	0	0	0	0	0	0	0	0	0
401		0	0	0	0	0	0	0	0	0	0	0	0
402		0	0	0	0	0	0	0	0	0	0	0	0
403		0	0	0	0	0	0	0	0	0	0	0	0
404		0	0	0	0	0 ^s	0	0 ^s	0	0	0	0	0 ^s
405		0	0	0	0	0	0	0	0	0	0	0	0
406		0	0	0	0	0	0	0	0	0	0	0	0
407		0	0	0	0	0	0	0	0	0	0	0	0
408		0	0	0	0	0	0	0	0	0	0	0	0
409		0	0	0	0	0 ^s	0	0 ^s	0	0	0	0	0
410		0	0	0	0	0	0	0	0	0	0	0	0

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R.T. = 24C

Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
435		0	0	0	0	0	0	0	0	0	0	0	0
436		0	0	0	0	0	0	0	0	0	0	0	0
437		0	0	0	0	0	0	0	0	0	0	0	0
438		0	0	0	0	0	0	0	0	0	0	0	0
439		0	0	0	0	0	0	0	0	0	0	0	0
440		0	0	0	0	0	0	0	0	0	0	0	0
441		0	0	0	0	0	0	0	0	0	0	0	0
442		0	0	0	0	0	0	0	0	0	0	0	0
443		0	0	0	0	0	0	0	0	0	0	0	0
444		0	0	0	0	0	0	0	0	0	0	0	0
445	VANN	0	0	0	0	0	0	0	0	0	0	1/2	0 ⁵
446		0	0	0	0	0	0	0	0	0	0	0	0
447		0	0	0	0	0	0	0	0	0	0	0	0
448		0	0	0	0	0	0	0	0	0	0	0	0
449		0	0	0	0	0	0	0	0	0	0	0	0
450		0	0	0	0	0	0	0	0	0	0	0	0
451		0	0	0	0	0	0	0	0	0	0	0	0
452		0	0	0	0	0	0	0	0	0	0	0	0
453		0	0	0	0	0	0	0	0	0	0	0	0
454		0	0	0	0	0	0	0	0	0	0	0	0
455		0	0	0	0	0	0	0	0	0	0	0	0
456		0	0	0	0	0	0	0	0	0	0	0	0
457		0	0	0	0	0	0	0	0	0	0	0	0
458		0	0	0	0	0	0	0	0	0	0	0	0
459		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	I
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LI
460		0	0	0	0	0	0	0	0	0	0	0	
461		0	0	0	0	0	0	0	0	0	0	0	
462		0	0	0	0	0	0	0	0	0	0	0	
463		0	0	0	0	0	0	0	0	0	0	0	
464		0	0	0	0	0	0	0	0	0	0	0	
465		0	0	0	0	0	0	0	0	0	0	0	
466		0	0	0	0	0	0	0	0	0	0	0	
467		0	0	0	0	0	0	0	0	0	0	0	
468		0	0	0	0	0	0	0	0	0	0	0	
469		0	0	0	0	0	0	0	0	0	0	0	
470		0	0	0	0	0	0	0	0	0	0	0	
471		0	0	0	0	0	0	0	0	0	0	0	
472	MILL	1	0	0	0	0	0	0	0	1	0	1/2	0
473		0	0	0	0	0	0	0	0	0	0	0	
474		0	0	0	0	0	0	0	0	0	0	0	
475		0	0	0	0	0	0	0	0	0	0	0	
476	NIEL	0	0	0	0	0	0	0	0	1	0	0	
477		0	0	0	0	0	0	0	0	0	0	0	
478		0	0	0	0	0	0	0	0	0	0	0	
479		0	0	0	0	0	0	0	0	0	0	0	
480		0	0	0	0	0	0	0	0	0	0	0	
481		0	0	0	0	0	0	0	0	0	0	0	
482		0	0	0	0	0	0	0	0	0	0	0	
483		0	0	0	0	0	0	0	0	0	0	0	

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24°C

		RT	15' (24C)	30' 37C		IAT(inch)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
484	BAKE	0	0	0	0	0	0	0	0	1	0	0	0
485		0	0	0	0	0	0	0	0	0	0	0	0
486		0	0	0	0	0	0	0	0	0	0	0	0
487		0	0	0	0	0	0	0	0	0	0	0	0
488		0	0	0	0	0	0	0	0	0	0	0	0
489		0	0	0	0	0	0	0	0	0	0	0	0
490		0	0	0	0	0	0	0	0	0	0	0	0
491		0	0	0	0	0	0	0	0	0	0	0	0
492		0	0	0	0	0	0	0	0	0	0	0	0
493		0	0	0	0	0	0	0	0	0	0	0	0
494		0	0	0	0	0	0	0	0	0	0	0	0
495		0	0	0	0	0	0	0	0	0	0	0	0
496		0	0	0	0	0	0	0	0	0	0	0	0
497		0	0	0	0	0	0	0	0	0	0	0	0
498		0	0	0	0	0	0	0	0	0	0	0	0
499		0	0	0	0	0	0	0	0	0	0	0	0
500		0	0	0	0	0	0	0	0	0	0	0	0
501		0	0	0	0	0	0	0	0	0	0	0	0
502		0	0	0	0	0	0	0	0	0	0	0	0
503		0	0	0	0	0	0	0	0	0	0	0	0
504		0	0	0	0	0 ^s	0 ^s	0 ^s	0 ^s	0	0	0 ^s	0
505		0	0	0	0	0	0	0	0	0	0	0	0
506	NEWB	0	0	0	0	0	0	0	0	(1)	0	1/2	(1/2)
507		0	0	0	0	0	0	0	0	0	0	0	0

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Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		15'		30'		IAT (imm)		5'		15'		10'		IAT		IAT	
		(24C)		37C				IAT		RT		37C				(5C)	
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS				
508		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
509		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
510		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
511		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
512		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
513		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
514		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
515		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
516		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
517		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
518		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
519		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
520		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
521		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
522		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
523		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
524		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
525		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
526		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
527		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
528		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
529		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
530		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
531		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Sal cells: washed 3x in saline 5%
 LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		15' (24C)		30' 37C		IAT(imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
532		0	0	0	0	0	0	0	0	0	0	0	0
533		0	0	0	0	0	0	0	0	0	0	0	0
534		0	0	0	0	0	0	0	0	0	0	0	0 ^s
535		0	0	0	0	0	0	0	0	0	0	0	0
536		0	0	0	0	0	0	0	0	0	0	0	0
537		0	0	0	0	0	0	0	0	0	0	0	0
538		0	0	0	0	0	0	0	0	0	0	0	0
539		0	0	0	0	0	0	0	0	0	0	0 ^s	0 ^s
540		0	0	0	0	0	0	0	0	0	0	0	0
541		0	0	0	0	0	0	0	0	0	0	0	0
542		0	0	0	0	0	0	0	0	0	0	0	0
543		0	0	0	0	0	0	0	0	0	0	0	0
544		0	0	0	0	0	0	0	0	0	0	0	0
545		0	0	0	0	0	0	0	0	0	0	0	0
546		0	0	0	0	0	0	0	0	0	0	0	0
547		0	0	0	0	0	0	0	0	0	0	0	0
548		0	0	0	0	0	0	0	0	0	0	0	0
549		0	0	0	0	0	0	0	0	0	0	0	0
550		0	0	0	0	0	0	0	0	0	0	0	0
551		0	0	0	0	0	0	0	0	0	0	0	0
552		0	0	0	0	0	0	0	0	0	0	0	0
553		0	0	0	0	0	0	0	0	0	0	0	0
554		0	0	0	0	0	0	0	0	0	0	0	0
555		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
556		0	0	0	0	0	0	0	0	0	0	0	0
557		0	0	0	0	0	0	0	0	0	0	0	0
558		0	0	0	0	0	0	0	0	0	0	0	0
559		0	0	0	0	0	0	0	0	0	0	0	0
560	WALK	0	1	(1)	(1)	1	1	(1)	(1)	1	(1)	1 1/2	1
561		0	0	0	0	0	0	0	0	0	0	0	0
562		0	0	0	0	0	0	0	0	0	0	0	0
563		0	0	0	0	0	0	0	0	0	0	0	0
564		0	0	0	0	0	0	0	0	0	0	0	0
565		0	0	0	0	0	0	0	0	0	0	0	0
566		0	0	0	0	0	0	0	0	0	0	0	0
567		0	0	0	0	0	0	0	0	0	0	0	0
568		0	0	0	0	0	0	0	0	0	0	0	0
569		0	0	0	0	0	0	0	0	0	0	0	0
570		0	0	0	0	0	0	0	0	0	0	0	0
571		0	0	0	0	0	0	0	0	0	0	0	0
572		0	0	0	0	0	0	0	0	0	0	0	0
573		0	0	0	0	0	0	0	0	0	0	0	0
574		0	0	0	0	0	0	0	0	0	0	0	0
575		0	0	0	0	0	0	0	0	0	0	0	0
576		0	0	0	0	0	0	0	0	0	0	0	0
577		0	0	0	0	0	0	0	0	0	0	0	0
578		0	0	0	0	0	0	0	0	0	0	0	0
579		0	0	0	0	0	0	0	0	0	0	0	0
580		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT	15' (24C)	30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
581		0	0	0	0	0	0	0	0	0	0	0	0
582		0	0	0	0	0	0	0	0	0	0	0	0
583		0	0	0	0	0	0	0	0	0	0	0	0
584		0	0	0	0	0	0	0	0	0	0	0	0
585		0	0	0	0	0	0	0	0	0	0	0	0
586		0	0	0	0	0	0	0	0	0	0	0	0
587	WALK	0	0	0	0	0	0	0	0	0	0	1½	1½
588		0	0	0	0	0	0	0	0	0	0	0	0
589		0	0	0	0	0	0	0	0	0	0	0	0
590		0	0	0	0	0	0	0	0	0	0	0	0
591		0	0	0	0	0	0	0	0	0	0	0	0
592		0	0	0	0	0	0	0	0	0	0	0	0
593		0	0	0	0	0	0	0	0	0	0	0	0
594		0	0	0	0	0	0	0	0	0	0	0	0
595		0	0	0	0	0	0	0	0	0	0	0	0
596		0	0	0	0	0	0	0	0	0	0	0	0
597		0	0	0	0	0	0	0	0	0	0	0	
598		0	0	0	0	0	0	0	0	0	0	0	0
599		0	0	0	0	0	0	0	0	0	0	0	0
600		0	0	0	0	0	0	0	0	0	0	0	0
601		0	0	0	0	0	0	0	0	0	0	0	0
602		0	0	0	0	0	0	0	0	0	0	0	0
603		0	0	0	0	0	0	0	0	0	0	0	0
604		0	0	0	0	0	0	0	0	0	0	0	0

Sal cells: washed 3x in saline 5%
LISS cells: washed 3x in saline 1x in LISS 2%

SERA from: IHR

R.T. = 24C

		RT 15' (24C)		30' 37C		IAT (imm)		5' IAT		15' RT	10' 37C	IAT	IAT (5C)
		SAL	ALB	SAL	ALB	SAL	ALB	SAL	ALB	LISS	LISS	LISS	LISS
605		0	0	0	0	0	0	0	0	0	0	0	0
606		0	0	0	0	0	0	0	0	0	0	0	0
607		0	0	0	0	0	0	0	0	0	0	0	0
608		0	0	0	0	0	0	0	0	0	0	0	0
609		0	0	0	0	0	0	0	0	0	0	0	0
610		0	0	0	0	0	0	0	0	0	0	0	0
611		0	0	0	0	0	0	0	0	0	0	0	0
612		0	0	0	0	0	0	0	0	0	0	0	0
613		0	0	0	0	0	0	0	0	0	0	0	0
614		0	0	0	0	0	0	0	0	0	0	0	0
615		0	0	0	0	0	0	0	0	0	0	0	0
616		0	0	0	0	0	0	0	0	0	0	0	0
617		0	0	0	0	0	0	0	0	0	0	0	0
618		0	0	0	0	0	0	0	0	0	0	0	0
619		0	0	0	0	0	0	0	0	0	0	0	0
620		0	0	0	0	0	0	0	0	0	0	0	0
621		0	0	0	0	0	0	0	0	0	0	0	0
622		0	0	0	0	0	0	0	0	0	0	0	0
623		0	0	0	0	0	0	0	0	0	0	0	0
624		0	0	0	0	0	0	0	0	0	0	0	0
625		0	0	0	0	0	0	0	0	0	0	0	0
626		0	0	0	0	0	0	0	0	0	0	0	0
627		0	0	0	0	0	0	0	0	0	0	0	0
628		0	0	0	0	0	0	0	0	0	0	0	0

$$R.T. = 24^{\circ}C$$

SUMMARY OF RESULTS OF PART 5

One thousand and one sera from unselected hospital patients (sera from four different pathology laboratories) were tested against red cells from CPD donor segments by saline, albumin and LISS techniques at room temperature and 37C. The tests were initially set -up by incubating at room temperature, and then moved to 37C. All positive reactions at 37C were repeated using a pre-warmed technique at 37C. A total of 44 sera contained antibodies.

The following results were obtained:

	Number of Sera Reactions		
	Saline	Albumin	LISS

Room Temperature (24C) Agglutination	8	15	20
37C Agglutination	1	4	1
Indirect Antiglobulin Test (RT + 37C)	8	12	27
Indirect Antiglobulin Test (Pre-warmed 37C)	8	12	26

Agglutination at room temperature occurred in 20 sera (2%) in LISS as compared to 8 in saline (0.8%) and 15 (1.5%) in albumin. Indirect antiglobulin tests at 37C (with a 15 minutes of incubation at room temperature preceding the 37C incubation) were positive with 27 (2.7%) of the sera using LISS but only 12 (1.2%) using albumin and 8 (0.9%) using saline suspended red cells. When the tests were carried out at 37C without the room temperature incubation one less serum reacted by the LISS procedure.

Of the 44 reactive sera only 39 had sufficient quantity to test against panels.

Of the 39 sera tested 9 did not react with any panel cells and 13 showed blood group specificity.

The following specificities have so far been defined:

Agglutinins

- 1 anti-Le^a: Reacted only by LISS at room temperature.
- 1 anti-Le^b: Reacted by saline, albumin and LISS at room temperature.
- 1 anti-P₁: Reacted by saline, albumin and LISS at room temperature.
- 2 anti-N: Reacted by albumin and LISS only at room temperature.
- 1 anti-I: Reacted by LISS only at room temperature.
- 2 anti-I: Reacted by albumin only at room temperature
- 1 anti-I: Reacted by saline, albumin and LISS at room temperature.

Indirect Antiglobulin Test (IAT) at 37C

- 1 anti-D: Reacted by saline, albumin, and LISS in the IAT.
- 1 anti-E: Reacted by saline, albumin, and LISS in the IAT.
- 1 anti-K: Reacted by LISS only in the IAT.
- 1 ? anti-I, H or IH: Reacted by saline, albumin and LISS in IAT only.

The 26 antibodies not containing specific antibodies were retested x2 (different days). (It should be noted that the initial screening was performed against red cells from donor segments (CPD) and the repeat screenings were performed against Pfizer Panoscreen Reagent Red Cells and our own Bay Area Red Cell Panel.)

Only 13 of the 26 sera reacted when retested. None of these 13 showed a definable specificity by the tests performed. Of these:

- 7 reacted by LISS only.
- 2 reacted by saline, albumin and LISS.
- 3 reacted by albumin and LISS only.
- 1 reacted by saline and LISS only.